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Abiotic and Biotic TNT Transformations

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1. Introduction
2. Objectives
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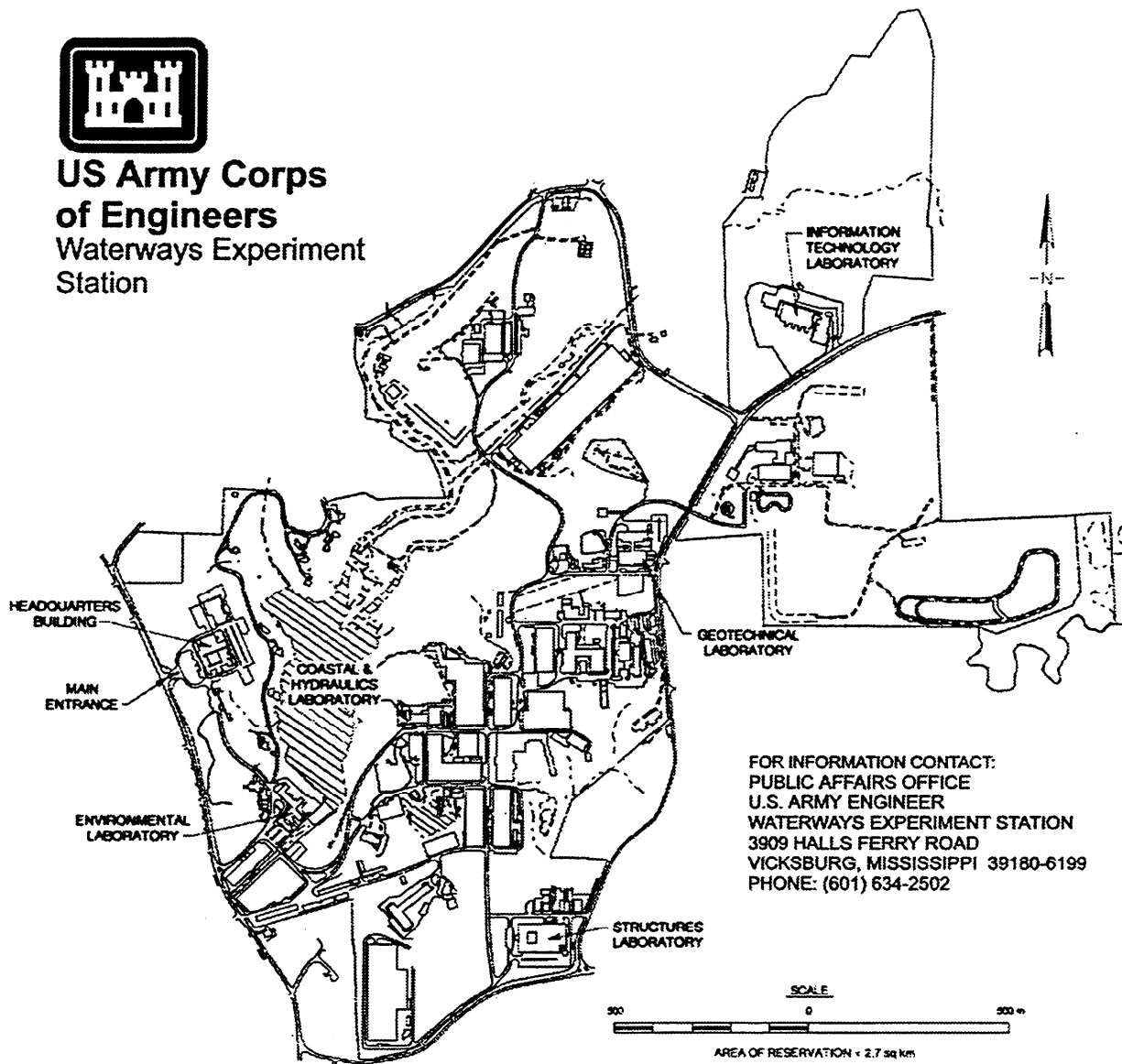
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Preface

The studies reported herein were conducted by the Environmental Laboratory (EL), U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS. The research was conducted under the Environmental Quality Basic Research Program. Dr. M. John Cullinane, WES, was the Program Manager.

Personnel who cooperated in the execution of the study and preparation of this report include Ms. Cynthia B. Price and Dr. James M. Brannon, Ecosystem Processes and Effects Branch (EPEB), Environmental Processes and Effects Division (EPED), EL; and Ms. Charolett A. Hayes, AScl Corporation. The authors acknowledge Mr. Rory Frederick and Ms. Toni Neumann for technical assistance. Technical reviews were provided by Drs. Douglas Gunnison and Judith C. Pennington, EPEB, EPED.

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Introduction

Many U.S. Army and other Department of Defense (DoD) installations contain soil and groundwater contaminated with the explosive 2,4,6-trinitrotoluene (TNT). Subsurface and surface contamination with TNT is often associated with munitions manufacturing, and with loading, assembling, and packing facilities (Pugh 1982; Spaulding and Fulton 1988).

The major factors affecting fate and transport of TNT in the subsurface are transformation, sorption, and irreversible soil binding (Townsend and Myers 1996). However, interpretation of sorption data for explosives, especially TNT, is confounded by the formation of TNT transformation products such as 4-amino-2,6-dinitrotoluene (4ADNT), 2-amino-4,6-dinitrotoluene (2ADNT), 2,4-diamino-6-nitrotoluene (2,4DANT), 2,6-diamino-4-nitrotoluene (2,6DANT), and trinitrobenzene (TNB) (Kaplan and Kaplan 1982). Only recently have TNT reductive transformation products been measured in laboratory and field studies (Townsend, Myers, and Adrian 1995; Price, Brannon, and Hayes in preparation; Comfort et al. 1995; Selim, Xue, and Iskandar 1995; Xue, Iskandar, and Selim 1995; Haderlein, Weissmahr, and Schwarzenbach 1996). TNT transformations are significantly enhanced under anaerobic conditions (Price, Brannon, and Hayes in preparation).

TNT transformations in soils can occur both biologically and abiotically. When sterilized and unsterilized soils were used (Pennington and Patrick 1990; Myers et al. In preparation), transformations were observed under both conditions, suggesting the presence of a purely abiotic transformation component. Transformations were far more extensive in unsterilized soils than in sterilized soils. Abiotic reduction of nitroaromatics other than TNT to amino products by Fe^{+2} has been shown to occur (Heijman et al. 1993, Heijman et al. 1995, and Haderlein and Schwarzenbach 1995). TNT has been demonstrated to disappear in the presence of montmorillonite or kaolinite when Fe^{+2} is added (Price, Brannon, and Hayes in preparation), but the transformation products were not identified.

The objectives of this study were to demonstrate the presence of the Fe^{+2} abiotic reduction pathway in soils, investigate the pH dependence of the process, and investigate the relationship between aerobic TNT transformations and soil components.

2 Materials and Methods

Aerobic TNT Transformation Capacity of Soils

This experiment examined not only the capacity of uncontaminated aerobic soils to transform added TNT but also the soil components that affected TNT transformations. The study used sequential challenges of 9 biotic soils in duplicate with fresh aliquots of 100 mg/L TNT solutions. The following soils were used in the tests:

China Lake A
Socorro P
Socorro S
Picatinny B
Yuma 1-A
Yuma 2-B
BRL-SAS A
Yokena clay
WES reference soils

The test soils provided a wide range of physical and chemical characteristics (Table 1, Pennington et al. 1994). For example, total organic carbon (TOC) ranged from 176 to 24,010 mg/kg, cation exchange capacity (CEC) from 3.5 to 38.9 meq/100 g, and total iron from 9,597 to 30,900 mg/kg.

Each batch sorption experiment consisted of 20 g of soil and 80 mL of distilled water. Following soil and water addition to centrifuge tubes, the slurry was amended with 250 μ L of a TNT spike solution containing 32,000 mg TNT/L in methanol to yield an initial solution concentration of 100 mg TNT/L. The amended slurry was shaken on a reciprocating shaker in the dark at 100 excursions per minute for 72 hr, centrifuged at 6,500 \times g, after which the supernatant was removed for analysis. The solution phase was transferred to plastic bottles and frozen until analyzed. A fresh 100 mg/L TNT aliquot in 80 mL of distilled water was added and an additional 72-hr exposure cycle was conducted. After removing the solution phase from the third exposure cycle, the soils were mixed and analyzed for TNT, TNB, 2ADNT, 4ADNT, and total azoxytoluene [4,4',6,6'-tetranitro-2,

Table 1
Soil Chemical and Physical Characteristics

Soil	TOC ¹ (mg/kg)	% O.M. ²	pH	Salinity (ppt)	CEC ³ (meq/100g)	Conductivity (mS/cm)	Particle Size		
							% Sand	% Silt	% Clay
China Lake A ⁴	176	0.20	7.7	0	3.5	0.11	92.5	2.5	5.0
Socorro P ⁵	1,172	0.53	7.8	3	27.3	3.53	42.5	30.0	27.5
Socorro S ⁶	782	0.53	7.9	3	34.0	3.55	37.5	35.0	27.5
Picatinny B ⁷	6,344	2.23	5.0	0	9.8	0.15	62.5	32.5	5.0
Yuma 1-A	581	0.38	7.9	2	5.4	0.36	77.5	12.5	10.0
Yuma 2-B	363	0.22	7.9	7	14.1	6.5	75.0	17.5	7.5
BRL-SAS ⁸ A	10,536	2.5	6.0	0	10.7	0.336	18.0	68.0	14.0
Yokena clay	24,010	4.46	5.3	0	38.9	0.32	13.75	37.5	48.75
WES ⁹ reference soil	5,670	2.81	5.3	0	12.4	0.30	0	93.75	6.25

(Continued)

¹ TOC = total organic carbon; ² % O.M. = percent organic matter; ³ CEC = cation exchange capacity; ⁴ A = sample taken from soil surface; ⁵ P = sample taken from periphery of test pad; ⁶ S = sample taken from surface of test pad; ⁷ B = sample taken from below surface; ⁸ SAS = Sassafras Loam; ⁹ WES = Waterways Experiment Station in-house reference soil.

Table 1 (Concluded)					
Soil	Total Iron (mg/kg)	Oxalate Extractable Metals (mg/kg) ¹			
		A1	Ca	Fe	Mn
China Lake A	9,597	75.9	12.20	68.4	21.8
Socorro P	10,500	80.7	11.80	541.0	30.4
Socorro S	10,100	70.6	7.51	78.8	13.6
Picantlnny B	25,000	7,980.0	64.30	1,087.0	57.5
Yuma 1-A	15,000	85.7	7.06	231.0	12.4
Yuma 2-B	13,000	104.0	7.42	97.8	16.3
BRL-SAS A	15,000	253.0	2.85	478.0	127.0
Yokena clay	30,900	5,570.0	57.60	2,128.0	131.0
WES ^o reference soil	9,970	184.0	47.90	715.0	153.0
¹ A1 = aluminum; Ca = calcium; Fe = iron; Mn = manganese. (Pennington et al. 1994)					

2'-azoxytoluene (4,4AZOX) + 2,2',6,6'-tetrinitro-4,4'-azoxytoluene (2,2AZOX)] compounds.

Abiotic TNT Transformation

All operations were conducted in a glove bag under a nitrogen atmosphere. Prior to use, distilled-deionized water was sterilized by boiling and centrifuge tubes by autoclaving. Montmorillonite was not sterilized due to concerns about affecting Fe^{+2} sorbing surfaces. Previous experiments showed that neither sorption nor microbes potentially associated with the montmorillonite affected TNT test solution concentrations (Price, Brannon, and Hayes in preparation).

The effect of pH on TNT reduction in the presence of montmorillonite and Fe^{+2} was investigated at pH 6, 7, and 8. Two-hundred fifty mL of MES buffer (70 mmol 2-[N-morpholino]ethane-sulfonic acid, pH 5.5 to 6.7), or EPPS buffer (2-hydroxyethyl] piperazine-N-[3-propanesulfonic acid), pH 7.3 to 8.7), sufficient ferrous chloride to yield 75 mg Fe^{+2} /L (0.00134 moles Fe^{+2} /L), 100 mg of montmorillonite/L, and sufficient TNT in methanol (a few microliters) to give a solution concentration of 25 mg/L TNT (0.00011 mol/L) were added. Triplicate treatments were tumbled in a rotary tumbler at 32 rpm and sampled at 5-min., 60-min., and 24-hr intervals. The pH 8 experiment was repeated with sampling times of 1 min., 5 min., 10 min., 30 min., 60 min., and 24 hr. At each sampling period, sufficient EDTA (0.5 mM final concentration) was added to 10 mmol of test solution to stop the reaction (Heijman et al. 1993). The sample was frozen until analyzed. Samples were not filtered prior to extraction; therefore, concentration data include solution and montmorillonite phases combined. The pH of each solution was checked at each sampling time. Controls consisted of each buffer solution with TNT and Fe^{+2} but no montmorillonite. Controls were sampled after 24 hr of incubation.

The pH 7 experiment was repeated using the same procedures as in the pH8 experiment, except that samples were spiked with [U-ring- ^{14}C]2,4,6-TNT (Chemsyn Science Laboratories, Lenexa, KS) having a specific activity of 21.58 millicurie/mmol, a chemical purity > 98 percent as determined by high performance liquid chromatography (HPLC), and a radio chemical purity >98 percent as determined by radio-HPLC. Controls were run with montmorillonite + TNT and Fe^{+2} + TNT. Experiments were sampled at 5 min, 30 min, and 1, 2, 4, 6, and 24 hr, and counted in a Packard TriCarb 2500 Liquid Scintillation Analyzer (Packard Instruments, Inc., Meridan, CT).

TNT was obtained from Holston Army Ammunition Plant. The TNT transformation products 2ADNT, 4ADNT, 2,4DANT, 2,6DANT, 2,2AZOX, and 4,4AZOX were obtained from SRI International (Menlo Park, CA). The purity of all standards was 99 percent or greater.

Biotic TNT Transformation

Experiments were also conducted with a biotic soil having high clay content (Yokena clay) under controlled Eh/pH conditions as described elsewhere (Price, Brannon, and Hayes in preparation). The redox potential was -150 mV at pH 7. To inactivate abiotic transformations caused by reduced metals such as Fe^{+2} , 5.0 mMol ethylene diamine tetraacetic acid (EDTA) was added 5 min prior to the addition of 15 mg TNT/L. Experiments were conducted in triplicate with and without EDTA. Concentrations of EDTA were analyzed periodically and maintained at the initial concentration.

Samples were obtained at 5, 15, 30 min, and 1, 4, 24 hr, and 7 days. Soluble iron (Fe) and manganese (Mn) were measured in each reactor prior to and after adding EDTA or TNT (t_0 samples). When sampling slurries without added EDTA, EDTA was added after sampling to stop abiotic reactions. Soils were analyzed at the end of 7 days by EPA 846 Method 8330 (Environmental Protection Agency 1992).

3 Results and Discussion

Aerobic TNT Transformation Capacity of Soils

Concentrations of TNT remaining in solution generally increased following each exposure cycle (Figure 1). This is consistent with TNT column studies (Townsend, Myers, and Adrian 1995) which showed increasing concentrations of TNT in column eluates over time. Concentrations of 2ADNT and 4ADNT generally either increased slightly or remained constant in the aqueous phase over the course of the three exposure cycles (Figure 1). Increasing exposure time resulted in increased production of the transformation products. Depletion of added TNT from the solution phase was highest during the initial exposure cycle, then decreased during subsequent cycles. This occurred whether initial TNT depletion was high, as was the case for Yokena clay and WES reference soils, or low, as was the case for BRL-SAS A and China Lake A. TNB was detected at low levels in the aqueous phase in two of the nine soils tested.

Analysis of the soils showed that high concentrations of total azoxy compounds were present in Socorro P, Yokena clay, and WES reference soils (Figure 2). High concentrations of 2ADNT and 4ADNT were also detected in the Yokena clay and WES reference soils (Figure 2).

Mass balances of the added TNT ranged from 34.3 percent to 100 percent (Figure 3) and were inversely related to oxalate-extractable manganese ($r^2=0.54$), percent organic matter ($r^2=0.54$), and extractable total azoxy compounds ($r^2=0.699$). While recovery of total azoxy compounds from soils increased as recovery of TNT decreased, it did not account for all the TNT added to the soils. Incomplete recovery of added TNT was probably due to the presence of unextracted or unanalyzed transformation products or binding to soil organic matter in a manner that renders it unextractable by the solvent (Pennington et al. 1995; Price, Brannon, and Hayes in preparation).

These results indicate that the ability of uncontaminated soils to adsorb and transform added TNT is highly dependent upon the nature of the soil. The fate of the added TNT is related to the inorganic chemistry and organic-matter content of the soils. If the soils are capable of removing TNT, losses of TNT continue as fresh TNT is introduced, although at a reduced rate.

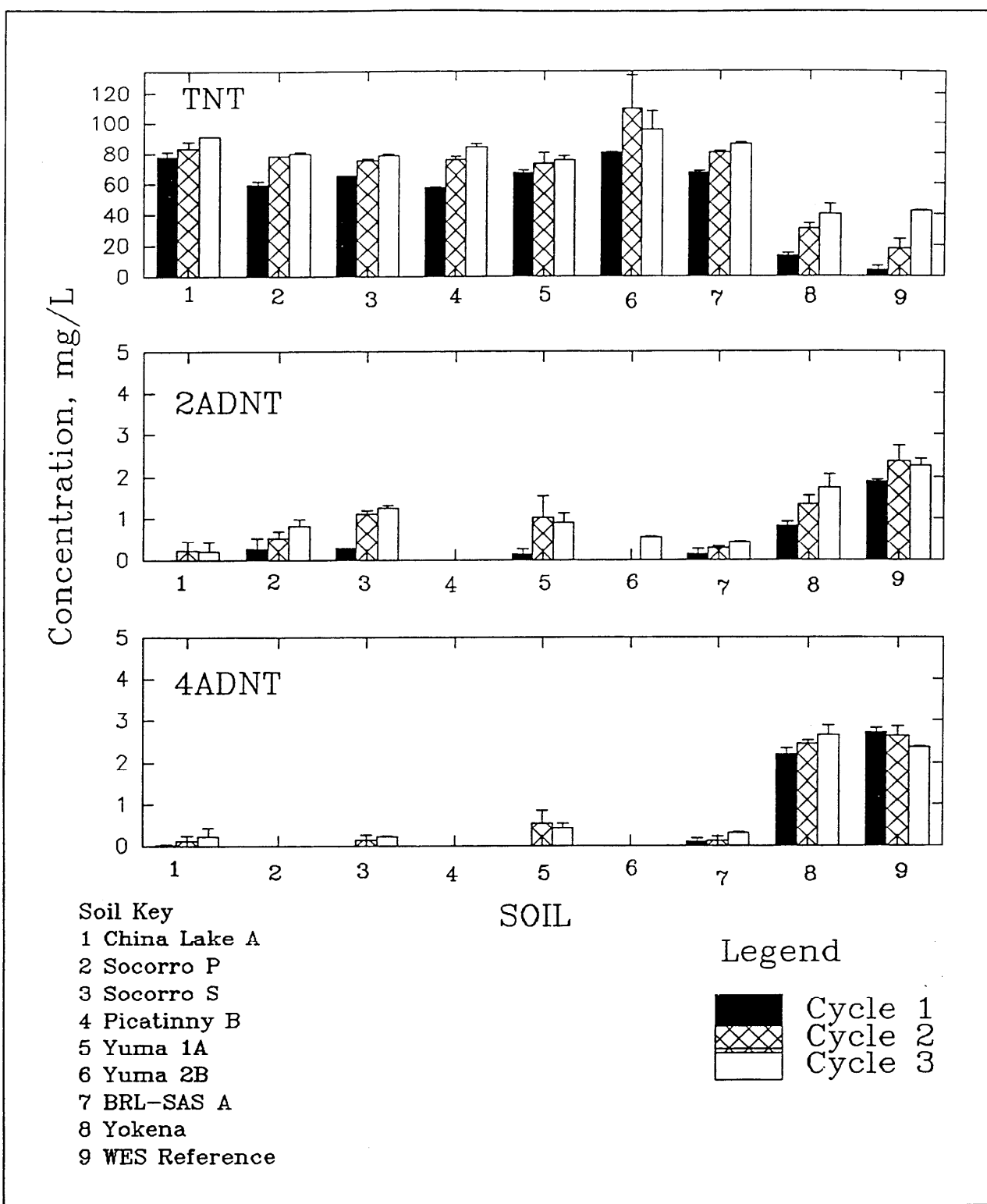


Figure 1. Solution-phase concentrations of TNT, 2ADNT, and 4ADNT following each exposure cycle

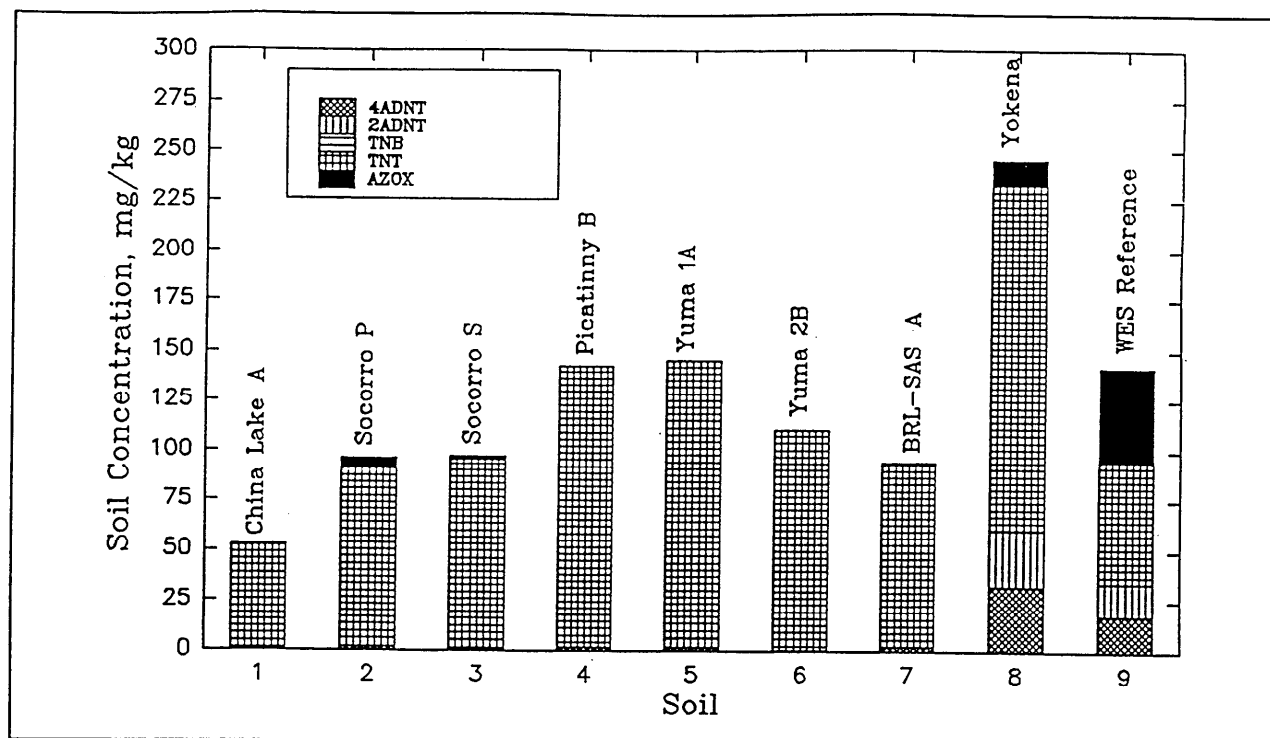


Figure 2. Soil concentrations [mg/kg dry weight (standard error)] of TNT and its transformation products following three exposure cycles

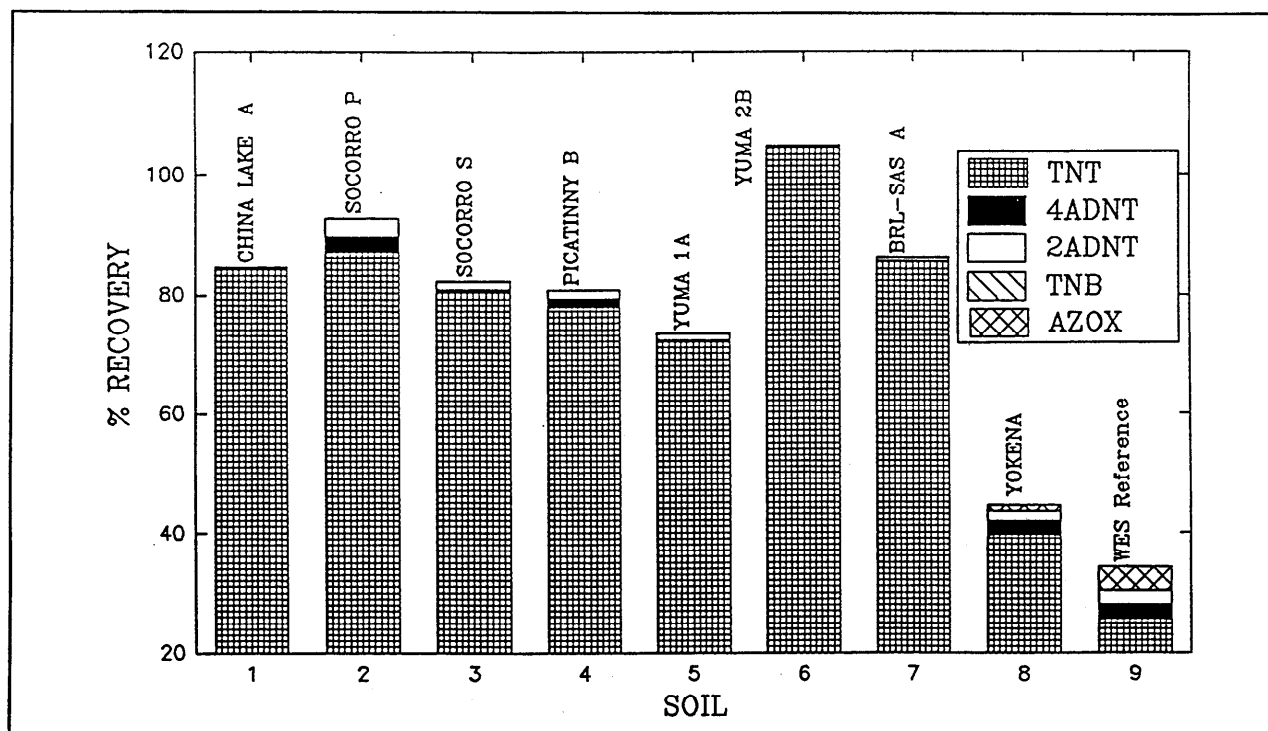


Figure 3. Percent recovery of TNT and transformation products from uncontaminated aerobic soils plus solution by HPLC following sequential addition of TNT

Abiotic TNT Transformation

Pronounced abiotic reduction of TNT at pH 6, 7, and 8 was observed in the montmorillonite + TNT + Fe^{+2} systems (Figure 4). Concentrations of TNT in the controls containing only Fe^{+2} and TNT were relatively invariant, averaging 20 ± 0.6 mg/L. Increasing pH was paralleled by decreasing concentrations of TNT at the end of 24 hr. After 24 hr at pH 7 and 8, substantial amounts of 2ADNT and 4,4AZOX were formed. At pH 8, substantial concentrations of 2,2AZOX were also detected (Figure 4). This is not surprising since formation of azoxy compounds is favored at $\text{pH} > 7$ (Funk et al. 1993). Neither of the diamino compounds were detected at any pH.

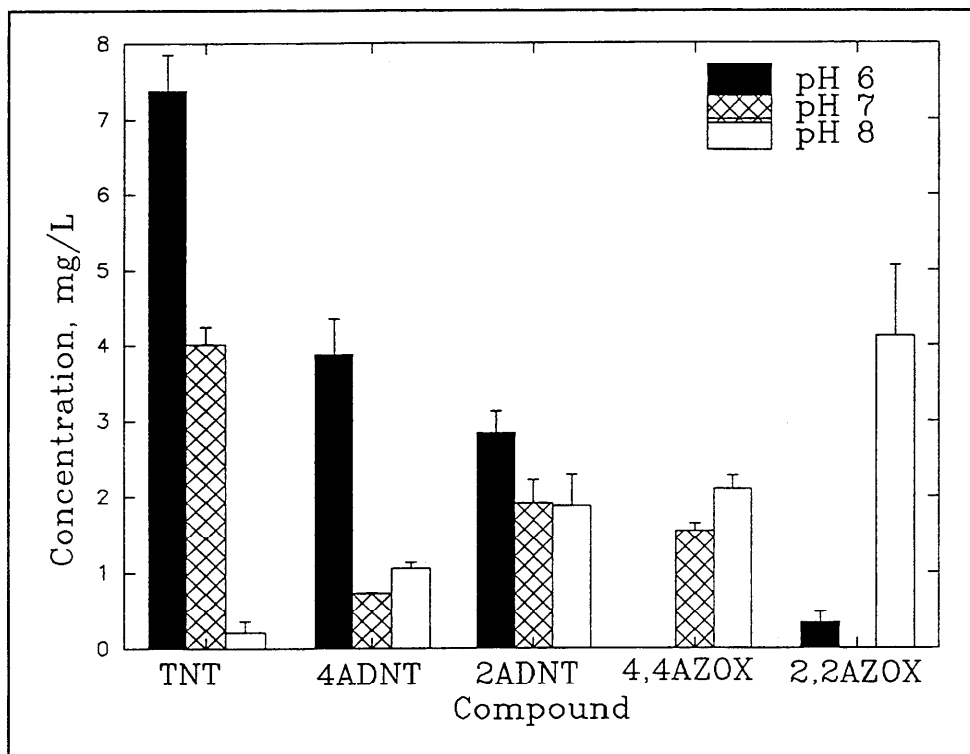


Figure 4. Solid plus solution-phase concentrations of TNT and its transformation products at pH 6, 7, and 8 following 24-hr exposure to Fe^{+2} and montmorillonite

The repetition of the pH 8 experiment with increased sampling times produced similar transformation products as in the initial experiment (Figure 5). The main difference was that only 4,4AZOX was found in the repeat experiment, whereas both 2,2AZOX and 4,4AZOX were found in the previous experiment.

At pH 6, 51.6 percent of the added TNT was recovered within 24 hr as either TNT or its transformation products, 4ADNT and 2ADNT (Table 2). Recoveries at pH 7 and 8 were substantially lower (Table 2). Half or more of the TNT disappearance was unaccounted for by the measured transformation products. The formation of unextractable or unknown transformation products appears to be responsible for

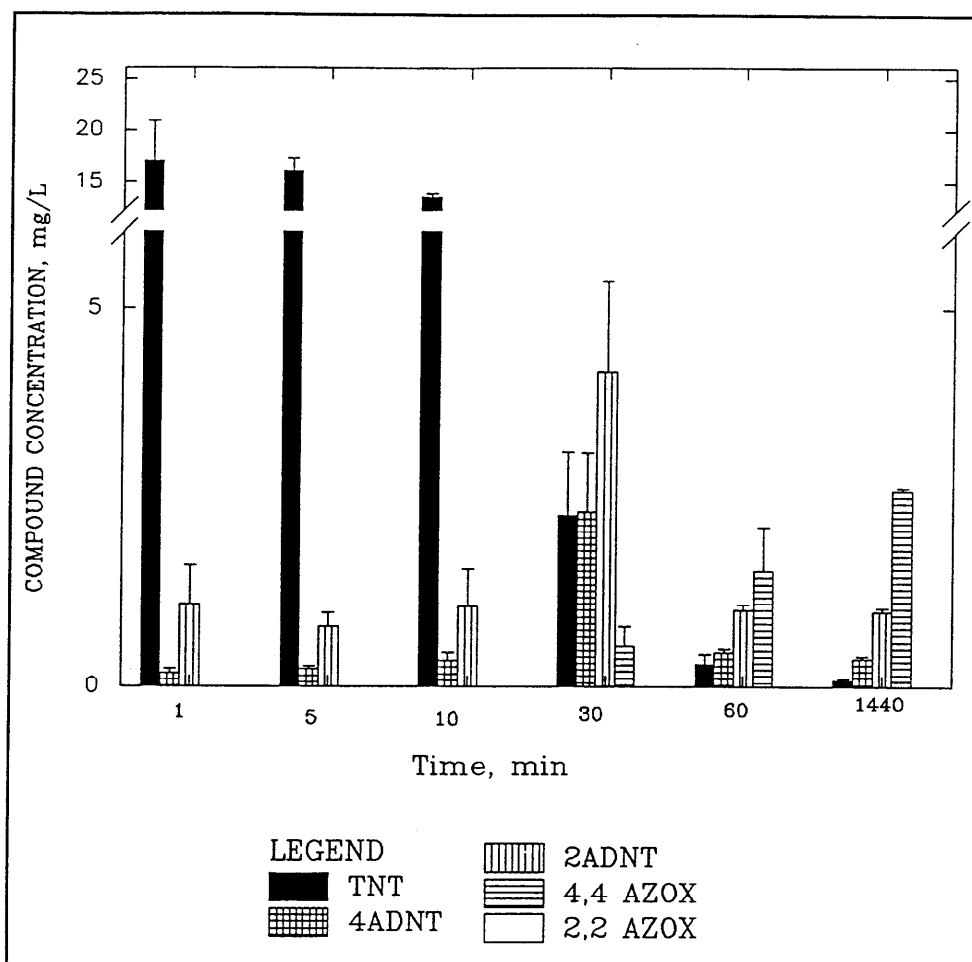


Figure 5. Solid plus solution-phase concentrations of TNT and its transformation products in the presence of Fe^{+2} and montmorillonite at pH8

Table 2
Percent Recovery [mean (standard error)] of TNT and Transformation Products

pH	Time		
	5 min	60 min	24 hr
6	53.2 (1.7)	44.5 (5.5)	51.6 (1.7)
7	77.6 (6.2)	71.4 (4.4)	29.7 (0.02)
8	48.1 (8.3)	31.0 (1.0)	33.9 (2.2)

Note: Number in () is the standard error.

poor recoveries. Soil organic matter was not present to bind the TNT in unextractable forms, and TNT did not irreversibly adsorb to the montmorillonite (Price, Brannon, and Hayes in preparation). Results of radioisotope studies (Figure 6) showed good recoveries, indicating that ^{14}C TNT was not lost from the test. Therefore, unextractable or unknown transformation products must account for the poor mass balance in the experiments where radio-labeled ^{14}C was not used.

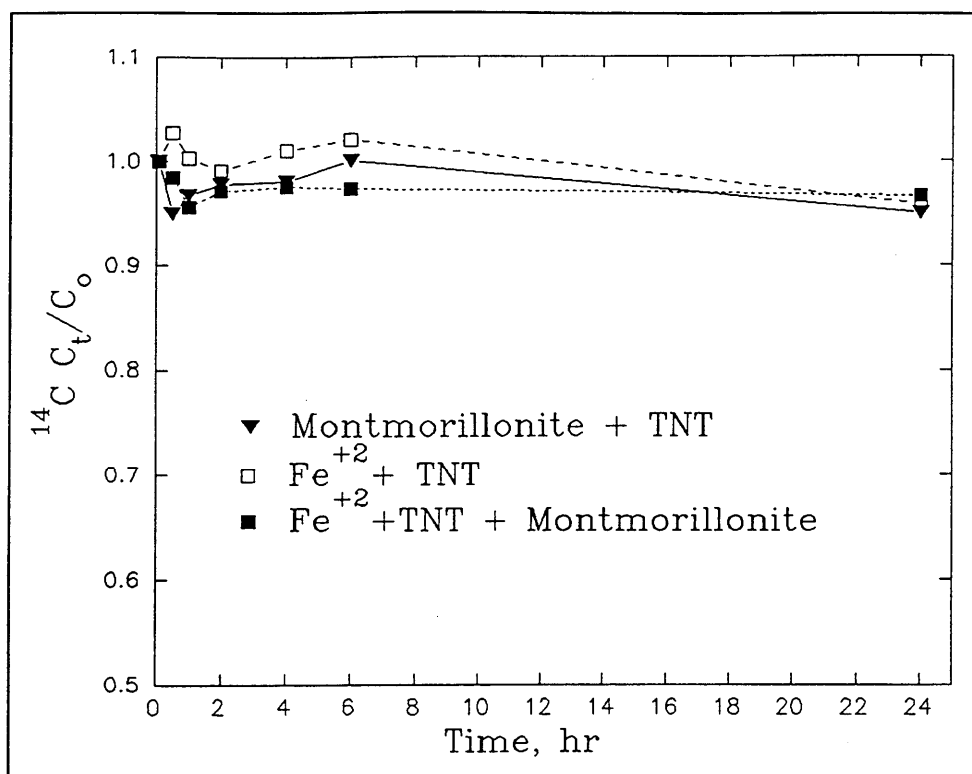


Figure 6. ^{14}C in solid plus solution phase over time at each treatment versus disintegrations/min/mL of ^{14}C at sample time (C_t) over initial disintegrations/min/mL (C_o)

Processes that remove TNT from solution can be expressed by pseudo first-order kinetics which take the form

$$dc/dt = -kc \quad (1)$$

where

c = chemical concentration of the reacting substance

k = pseudo first-order reaction constant

t = time

Pseudo first-order kinetics reduces to the equation

$$\ln (c_o/c) = kt \quad (2)$$

where

c_o = concentration of the reacting substance at time 0. Once a value of k is obtained, the half-life period of the reacting substance, $t_{1/2}$ can be calculated using the equation

$$k = 0.693/t_{1/2} \quad (3)$$

(Fair, Geyer, and Okun 1968). To quantify the rates of disappearance, concentrations of TNT and metabolite in the test were fit to the pseudo first-order kinetic equation. Observed data generally conformed well to pseudo first-order kinetics. At pH 7, the removal-rate constant was 0.072 hr^{-1} with an $r^2 = 0.97$, substantially slower than the removal rate at pH 8 which ranged from 4.36 to 4.81 hr^{-1} in two separate determinations (Table 3). Doubling the number of sampling times in the pH 8 repeat experiment did not have a pronounced effect on the TNT disappearance-rate constant or half-life. Half-life of TNT in the abiotic TNT + Fe^{+2} + montmorillonite system decreased as pH increased (Table 3).

Table 3
Summary of Half-lives and
Disappearance-rate Constants (K) for
Abiotic TNT Reduction

pH	K, hr^{-1}	r^2	$t_{1/2}$, hr
6	0.011	0.88	64
7	0.072	0.97	9.6
8	4.81	0.99	0.14
8*	4.36	0.99	0.16

* Repeated with increased sampling.

The reduction and removal of TNT from solution were more rapid and complete as pH increased. One possible reason is that the oxidation of Fe^{+2} to $\text{Fe}(\text{OH})_3$ is easier at pH 8 than at pH 6 or 7. Electron potential differences (E) for the oxidation reaction, calculated from the Nernst equation (Krauskopf 1967) for the reaction $\text{Fe}^{+2} + 3\text{H}_2\text{O} \rightleftharpoons \text{Fe}(\text{OH})_3 + 3\text{H}^+ + e^-$ are -0.18 V at pH 8 and 0.18 V at pH 6 (assuming

25°C and that the concentration of all reactants are unity). The lower the value of E, the more likely the reaction is to occur (Krauskopf 1967); therefore, at pH 8 oxidation of Fe^{+2} and the generation of electrons for TNT reduction is thermodynamically preferred.

Biotic TNT Transformation

The presence of the abiotic Fe^{+2} reduction mechanism for TNT in biotic soils was verified (Figure 7). Soluble iron showed a fourfold increase 30 min after the addition of 5.0 mMol EDTA, from 1.3 mg/L to 5.6 mg/L . Soluble manganese, another potential electron donor already present in the soil, increased from 1.4 mg/L to 5.2 mg/L . The EDTA resulted in a marked decrease in TNT disappearance during the first hour of exposure; however, disappearance resumed even though analyses revealed that EDTA concentrations had not decreased. This was probably due to Fe^{+2} dissociation from the EDTA complex resulting in abiotic reduction by Fe^{+2} , the presence of other abiotic systems, or microflora in the biotic soil acting to remove TNT from the solution. As a result, TNT in the anaerobic soil was unstable over the short-term (seven days) even with the Fe^{+2} abiotic transformation pathway suppressed.

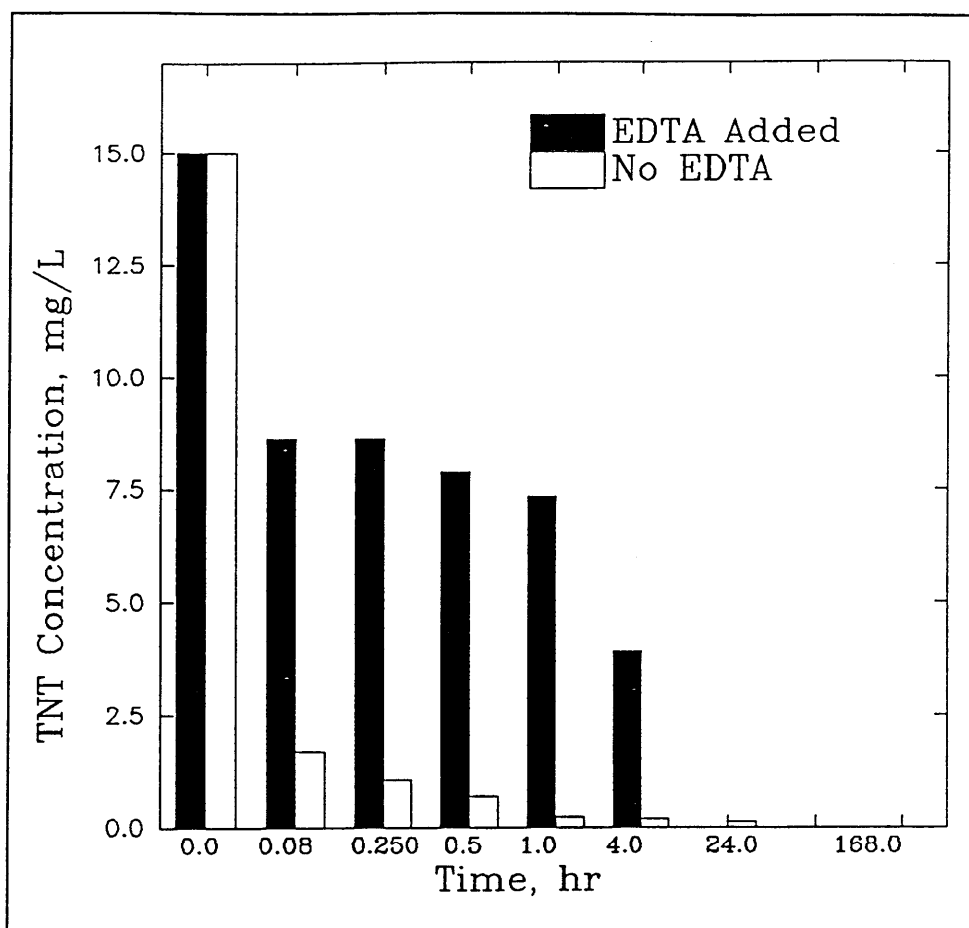


Figure 7. TNT concentrations in anaerobic soil slurry (-150 mV) in the presence and absence of EDTA

The presence of EDTA in the anaerobic soil slurry also suppressed the formation of TNT transformation products (Figure 8). The monoamino and diamino transformation products, 2ADNT and 2,4DANT, appeared at the same time in the EDTA and no-EDTA treatments, but persisted longer at significantly lower concentrations in the EDTA treatment. Concentrations of 4ADNT and 2,6DANT showed similar trends (data not shown). Concentrations of TNT and its transformation products in soil (Figure 9) following 7 days of incubation were generally higher in the EDTA treatment.

Results indicated that abiotic Fe^{+2} reduction of TNT occurred in both abiotic Fe^{+2} + montmorillonite systems and biotic soils. The abiotic reduction is pH sensitive with rates increasing as pH increases. Suppression of the abiotic pathway in soils slowed initial TNT reduction and the production of transformation products. Suppression, however, has only a transitory effect because TNT concentrations in soil with suppression are similar to soil without suppression after 24 hr. Even in a simple abiotic Fe^{+2} + montmorillonite system, recovery of TNT and analyzable transformation products were low, suggesting the formation of unextractable or unknown transformation products which was verified by $[^{14}\text{C}]$ TNT results.

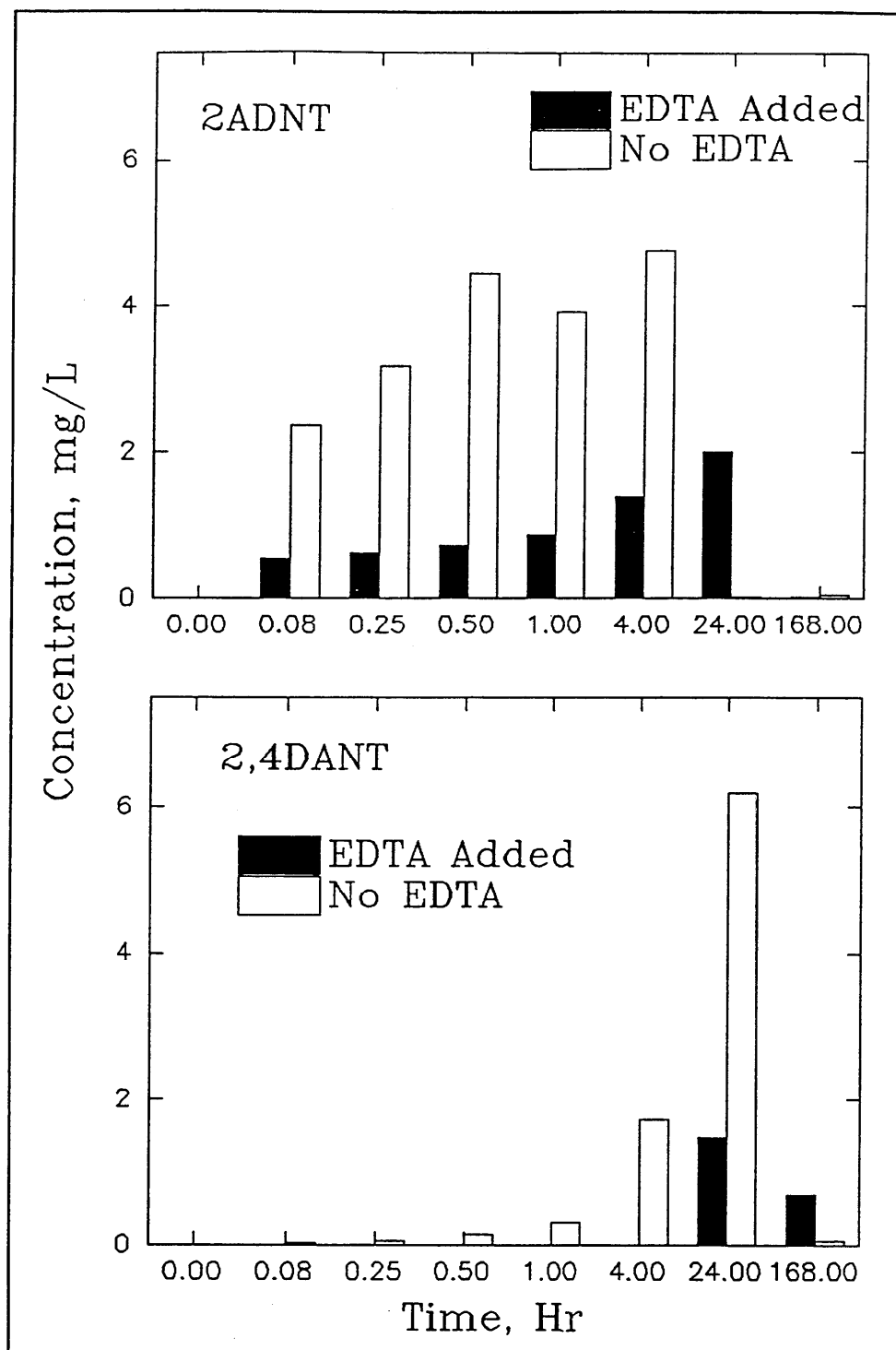


Figure 8. Concentrations of TNT transformation products in an anaerobic soil slurry (-150mV) in the presence and absence of EDTA

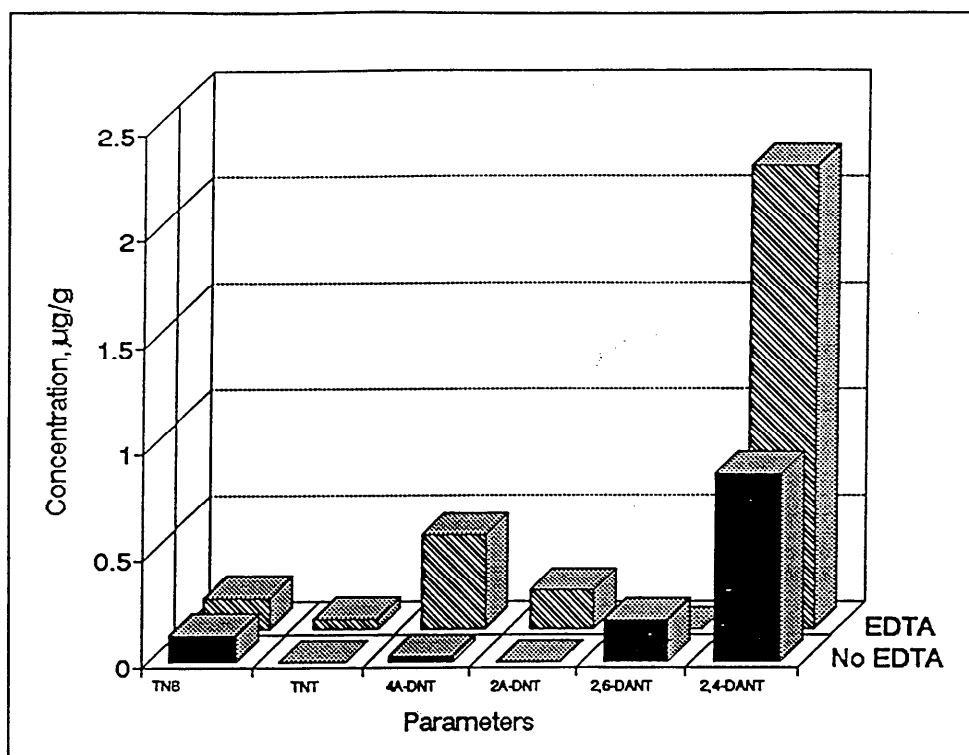


Figure 9. Soil concentrations of TNT and its transformation products in an anaerobic soil slurry (-150mV) in the presence and absence of EDTA following 7 days of incubation

4 Conclusions

A complete mass balance of TNT and its transformation products in even a simple abiotic Fe^{+2} + montmorillonite system was not attained. Low recoveries were due to the formation of unextractable or unknown transformation products. More research is needed to determine the identity of these products.

Abiotic and biotic mechanisms operate concurrently in soils to regulate TNT reduction. Processes that affect either the abiotic or biotic mechanisms can influence TNT reduction rates. Soil characteristics and environmental conditions exerted a marked influence on TNT fate and transformation under aerobic and anaerobic conditions. Increasing pH in anaerobic soils is likely to increase the TNT reduction rate because abiotic reduction of TNT by Fe^{+2} increases as pH increases. Suppression of either the abiotic or biotic mechanisms will lower the rate of TNT reduction.

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13. ABSTRACT (Maximum 200 words) Cleanup of soils and groundwater contaminated with the explosive TNT is a major concern. Interpretation of fate processes, especially equilibrium partitioning, is confounded by the ready transformation of TNT to mono and diamino products. The objectives of this study were to demonstrate the presence of the Fe ⁺² abiotic reduction pathway in soils, investigate the pH dependence of the process, and investigate the relationship between aerobic TNT transformations and soil components. Effects of pH on abiotic transformations of TNT were examined in buffered batch tests with montmorillonite and Fe ⁺² . Results indicated that TNT was rapidly reduced to monoamino transformation products under abiotic conditions, with the rate of reduction increasing as pH increased. Recoveries of unlabeled TNT and transformation products ranged from 30 to 50 percent, but radioisotope tests showed complete recovery of ¹⁴ C, indicating that significant quantities of unextractable or unknown transformation products were produced. Until these transformation products are identified, mass balances for TNT will not be possible, even in simple environmental systems without the use of ¹⁴ C. (Continued)				
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Suppression of the abiotic Fe^{+2} reduction mechanism in anaerobic soil by complexing Fe^{+2} with EDTA was short-term, lasting for only an hour before other mechanisms acted to reduce TNT to monoamino and diamino compounds. However, the abiotic Fe^{+2} reduction pathway accelerated the initial rate of TNT reduction in anaerobic soil. Suppression of either the abiotic or biotic mechanisms will lower the rate of TNT reduction.

Recoveries of added TNT were inversely related to oxalate extractable manganese, percent organic matter, and extractable total axes compounds in aerobic soil. This indicates that the nature of soils, in addition to environmental conditions, will impact TNT fate and transport.